Considerations for Evaluating Ultraviolet Radiation-induced Genetic Damage Relative to Antarctic Ozone Depletion

Deneb Karentz

University of San Francisco, San Francisco, California

Springtime ozone depletion over the Antarctic results in increased UVB in local marine environments. It has been established that decreases in primary productivity occur with decreases in ozone concentrations, but the impact of increased UVB on the functioning and stability of the ecosystem has not yet been determined. Very little has been done to evaluate the potential for genetic damage caused by the increase in UVB, and this type of damage is most significant relative to the fitness and maintenance of populations. An essential problem in evaluating genotoxic effects is the lack of appropriate techniques to sample and quantify genetic damage in field populations under ambient UVB levels. In addition, it is currently not feasible to estimate exposure levels for organisms in their natural habitats. — Environ Health Perspect 102(Suppl 12):61–64 (1994)

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Every spring for approximately the past 20 years, over 50% of the ozone in the stratosphere over the Antarctic disappears (*1–4*). At the surface of the earth, decreases in atmospheric ozone concentrations are manifested as increases in biologically harmful ultraviolet B radiation (UVB, 280–320 nm) (*5,6*). There are several aspects of Antarctic ozone depletion that raise important considerations relative to evaluating biologic and ecologic effects in the marine ecosystem (*7,8*):

- the springtime ozone depletion cycle is a recent event relative to geologic and evolutionary time scales,
- increases of UVB during spring coincide with the time that organisms are first experiencing solar radiation after the dark winter,
- springtime increases in UVB occur at the same time that many Antarctic organisms begin their reproductive cycles,
- there are no "pre-ozone depletion" data relating to the UV-photobiology of Antarctic organisms, and
- there is a significant lack of methodology to investigate and quantify *in situ* UV-induced genetic damage to aquatic organisms.

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Address correspondence to Dr. Deneb Karentz, Department of Biology, University of San Francisco, San Francisco, CA 94117-1080. Telephone (415) 666-2831. Fax (415) 666-2346.

UVB can affect many structural components and metabolic processes in aquatic organisms (*9–13*). Even without ozone depletion, UVB is a constant environmental stress, and ambient levels at any latitude can be potentially harmful to organisms. For this reason, increases in UVB stress caused by localized ozone depletion events are of major concern (*7,8,14*). During the past few years, ozone depletion in the Antarctic has exceeded the expected limits predicted from existing models (*15*). With recent observations of Arctic ozone depletion and projected continued declines in global ozone levels (*16,17*), increased understanding of the ecologic implications for aquatic systems is required (*18*).

UVB can penetrate to 60 m in Antarctic waters during spring (*19*); however, biologic effects have only been observed to approximately 20 m and most significant effects occur within the top 10 m (*19,20*). Ozone depletion effects on the Antarctic ecosystem have focused almost exclusively on phytoplankton, specifically the process of carbon fixation by photosynthesis (*19,21,22*). Since phytoplankton are the primary producers and alterations in net photosynthesis will have ramifications at all other trophic levels, both consumer and detritovore populations will ultimately be affected. In addition to their importance in the food web, phytoplanktonic organisms are unicellular and can reproduce asexually. Generation times are on the order of days. Therefore, responses to environmental stresses, such as increased UVB, will occur more rapidly than in larger, sexually reproducing organisms with seasonal and yearly generation times.

Although DNA is a primary lethal target for UVB and genetic damage is a major consideration in sustaining population fitness, there are limited data available on genetic damage in aquatic organisms (e.g., *23–31*), and even fewer data specific for Antarctic species (*32,33*). In addition, research conducted has been on laboratory-maintained populations; and in many cases, experimental irradiations are performed with artificial light sources that cannot be readily compared to ecologically relevant intensities or wavelength distributions. There is an immediate need for techniques to examine genotoxic damage in individual species from natural communities.

Traditional methods for studying natural phytoplankton communities rely on filtration of whole or size-fractionated volumes of water and treatment of the filtrate as a single sample. In the case of phytoplankton, this filtrate could represent ten or many more species and the individual species would have differential tolerances to UVB. Analyzed collectively, the species-specific aspect of variability is totally obscured. In laboratory investigations of cultured Antarctic phytoplankton, variations in UVB tolerance (based on growth rate) have been related to the amount of DNA damage induced. In addition, levels of DNA damage were closely correlated to cell size (*32*). Smaller species had higher concentrations of photoproducts in their DNA and were more sensitive to UVB than larger phytoplankton species.

The implications of size sensitivity to UVB among phytoplankton species could be very significant (*34*). If increased UVB in the environment selects for larger cells, then the size spectrum of the community

would gradually change. This type of shift to species with larger cell size has been observed in field populations of diatoms in a lotic system (*35*). Any alterations in the taxonomic structure and size distribution within microalgal communities could potentially alter the transfer of energy through the aquatic food web. This may be the most critical aspect of the ecologic consequences of ozone depletion; but there are no data available from natural populations under ambient UVB exposures to substantiate this hypothesis.

Many molecular techniques have been developed for investigating genetic characteristics of bacterial and mammalian cells. Some of these methods allow for detailed analyses of single cells or single chromosomes; however, the transfer of such technology to marine field studies on bacteria, algae, and invertebrates is often complicated by sampling techniques, the basic biology of organisms, and the presence of many extraneous compounds in water samples (e.g., the salt content of seawater can interfere with many assays).

Sampling schemes have to be carefully managed to prevent increased damage and/or increased repair during shipboard sampling procedures. For example, many phytoplankton studies are done by enclosing phytoplankton samples in containers of varying transparency to UV wavelengths and incubating at fixed depths to allow for ambient radiation exposures. In the process of lowering samples from the surface and retrieving samples from depth, there is the potential for intermittent or even prolonged exposure to sea surface and shipboard deck light intensities. Smith et al. (*19*) addressed this problem for carbon production experiments by devising a hinged deployment unit with a cloaking system that protects samples from sunlight once they are recovered from the water and brought onto the deck of the ship. Use of a similar system for DNA damage assessment would prevent excess damage caused by surface irradiation, but does not solve the problem of allowing dark repair processes to continue, altering the concentration of DNA photoproducts.

In addition to sampling techniques, marine organisms often have specific biologic characteristics that can interfere with DNA or chromosomal assays that are successfully used on mammalian and *Escherichia coli* cells. Many marine organisms have protective outer coverings (e.g., silicate frustules, chitinous exoskeletons) that interfere with DNA extraction and preparations for chromosomal analyses (*36*). Temperature is also a factor to consider. Antarctic organisms live at –2°C. The standard practice of placing mammalian cells on ice during DNA extraction procedures to prevent enzymatic digestion does not work. In one study, the isolation of high molecular weight DNA required for UV endonuclease assays was not successful in Antarctic marine diatoms. Instead, a radioimmunnoassay for DNA photoproducts was adopted (*32*). The radioimmunnoassay is a more complex and more sensitive method that can be effectively used on smaller DNA fragments.

A third consideration in the evaluation of genetic damage in marine organisms is the natural chemistry of seawater. The presence of dissolved and particulate matter can greatly interfere with methods that are routine for clonal cultures of mammalian and bacterial cells. The high salt concentration of seawater is often a major problem.

Despite some of these difficulties, methods for the evaluation of genotoxic, cellular, and developmental responses of Antarctic sea urchin embryos (*Sterechinus neumayeri*) to ambient UVB exposure have been successfully developed (*33*). These studies represent the first quantitative measurements of genetic damage in an endemic species under ambient UVB levels. Preliminary results indicate that ambient UVB can inhibit development of embryos and causes lethal damage to early life cycle stages. Exposure to UVB decreased the number of normally developed blastula, and increased the frequency of aberrant anaphases and under-developed and malformed embryos. Such impairment of development could cause alterations in the recruitment of animals into adult populations. Since many benthic organisms have planktonic larval stages, both benthic and pelagic communities can potentially be impacted.

Since Antarctic ozone depletion is seasonal and geographically restricted by the polar vortex, this region provides an excellent opportunity for field studies of UVB effects on marine organisms (*19*). However, extreme variability in the UVB exposure of organisms makes ecologic assessment difficult. The amount of UVB that reaches marine environments is dependent upon many factors. Ozone concentrations, clouds, and weather determine how much UVB reaches the sea surface. During spring, extensive areas of the Southern Ocean can be covered with ice and snow, reducing the penetration of UVB into the water column. In-water transmission is further affected by dissolved and particulate matter, including the density of organisms. All of these factors are constantly changing on short-term (daily) and long-term (seasonal) time scales.

One of the most problematic aspects of evaluating the biologic effects of ozone depletion is that it is very difficult to quantify the actual exposure of organisms in the water column. Incident UVB varies with season and time of day (*33*). Within the euphotic zone, UVB levels diminish with depth and the attenuation of UVB wavelengths is affected by a variety of hydrographic factors. The exposure of an individual organism is further dependent on its vertical positioning in the water column during daylight hours. Planktonic organisms are moved vertically through the water column by the motion of the water. The vertical mixing regime regulates the intensity and duration of the dose received. Some species, such as krill, undergo active vertical migrations that are usually related to feeding. Therefore, establishing the actual exposure level requires the consideration of elaborate interacting mechanisms relating to hydrology and behavior. Moreover, each species within the community can have a different time scale for vertical translocation.

The problem of determining exposure levels becomes even more confounded when one considers that higher wavelengths of solar radiation are required for photoenhanced repair (photoreactivation) of DNA damage (UVA, 320–400 nm) and for photosynthesis (photosynthetically active radiation PAR, 400–750 nm) to provide energy for nucleotide excision repair. The attenuation of UVB within the water column must be considered relative to the attenuation of UVA and PAR for any assessment of biologic effects, but very little is understood about the interaction of detrimental and beneficial wavelengths of solar radiation.

It is not yet possible to assess what the impact of the Antarctic ozone depletion cycle has been or will be on the Antarctic ecosystem. Changes have certainly been occurring since the very first depletion event. It is generally agreed that decreases in productivity and alterations in species composition are inevitable and even subtle changes in the quantity or quality of phytoplankton food sources could eventually affect larger Antarctic consumers such as penguins, seals, and whales. These kinds of changes are difficult to document on such a large scale as the Southern Ocean and research on the UV photobiology of Antarctic animals has not yet been initiated.

In addition to possible direct effects of UVB on energy transfer between trophic levels, ozone depletion has a high potential for inducing genetic damage in Antarctic organisms. However, *in situ* levels of genetic damage have not been determined. There is an

immediate need to develop suitable methods for measurement of genetic effects caused by ozone depletion. At this time, the ramifications of such damage relative to the course of natural selection in local populations is not known. With the total lack of baseline data on springtime UVB damage without ozone depletion, the impact of enhanced UVB cannot be properly evaluated. Until these types of data are available, it is not possible to determine if changes mediated by UVB damage will have a significant effect on the stability of the Antarctic ecosystem.

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